Am. J. Hum. Genet. 41:979–995, 1987

Genetics, Chance, and Morphogenesis

DAVID M. KURNIT,* WILLIAM M. LAYTON,† AND STEVEN MATTHYSSE‡

*Howard Hughes Medical Institute, University of Michigan Medical Center, Ann Arbor; †Dartmouth Medical School, Department of Anatomy, Hanover, NH; and ‡McLean Hospital, Harvard Medical School, Belmont, MA

SUMMARY

We posit that chance plays a major role in the occurrence of many common malformations that cluster in families but recur less frequently than expected for simple Mendelian traits. Once the role of random effects is accepted, the segregation of such malformations may be explained on the basis of Mendelian transmission of a single abnormal gene that predisposes to, but does not always result in, the abnormal phenotype. We apply a stochastic (probabilistic) single-gene model to the occurrence of malformations in mouse and man. The stochastic single-gene model suggests the feasibility of isolating individual genes that determine morphogenesis and sets limits on the precision with which the recurrence of malformations can be predicted.

THE THRESHOLD MODEL

Simple Mendelian unifactorial inheritance cannot explain the existence of diseases characterized by familial clustering with recurrence risks significantly <25%, unless the vague concept of "reduced penetrance" is invoked. Such inheritance patterns are often explained by means of a "multifactorial threshold" model (Penrose 1953; Edwards 1960; Carter 1965; Falconer 1965). The model proposes the existence of "an underlying gradation of some attribute immediately related to the causation of the disease" (Falconer 1965), with the attribute defining the liability of an individual to develop the disease. The curve of liability is continuous in both the general population and in relatives of affected cases, but the curve for relatives is shifted in the direction of a greater

Received April 8, 1987.

Address for correspondence and reprints: Dr. David Kurnit, Howard Hughes Medical Institute, 1150 West Medical Center Drive, MSRBI, Room 3520, Ann Arbor, MI 48109-0650.

propensity to develop the disease. When the liability exceeds a given threshold, the disease is manifested in an individual. The liability subsumes all factors, both intrinsic and extrinsic, that predispose to the occurrence of a disease. Hence the model is multifactorial, with the major factors assumed to be genetics and environment.

It was presumed that the multifactorial threshold model implies the involvement of many genes (polygenic inheritance) because the model "excludes situations where the variation of liability is discontinuous, which would apply to diseases determined by a single major gene" (Falconer 1965). Below we present computer simulations that show, on the contrary, that a stochastic single-gene model can yield continuous liability curves. On the basis of these simulations, we would modify the multifactorial hypothesis in two ways. First, we would include the role that chance plays during development, so that two individuals with identical genotypes and environments can have different phenotypes; a complete multifactorial model would include genetics, environment, *and* chance as factors (Newman 1985). Second, we would demonstrate that stochastic single-gene models describe adequately the quantitative specifications for which polygenic models were elaborated, with risk depending on the closeness and number of affected relatives.

A STOCHASTIC SINGLE-GENE MODEL FOR CARDIAC-SEPTAL DEFECTS

We performed computer simulations to describe endocardial cushion outgrowth and fusion (Kurnit et al. 1985*a*). In these simulations, randomly walking endocardial cells were allowed to migrate, divide, and adhere with probabilities set in advance. The random walk continued until all cells adhered to at least one neighbor and no further change was possible. Figure 1 describes cushion-to-cushion fusion, and figure 2 describes cushion-to-septum fusion. Technical and anatomic details are described in the legends to figures 1 and 2 and in Kurnit et al. (1985*a*).

The striking result was that variability in outcome was observed among multiple, independently performed simulations that used identical parameters. Figure 1 simulates endocardial cushion-to-cushion fusion; although the values of adhesion, migration, and division were identical in all four simulations, significant differences were observed in the final state, i.e., the intactness of the "septum" configured by the endocardial cells represented as dots in the outlined rectangles. Figure 2 simulates endocardial cushion-to-septum fusion; again, the parameters were identical in each of the four simulations, but the final outcomes differed. The first simulation in figure 2 shows normal, complete atrioventricular (AV) canal septation, whereas the other simulations in figure 2 manifest atrial (dorsal AV canal) and/or ventricular (ventral AV canal) septal defects (VSD). Figures 1 and 2 pictorialize how stochastic effects can yield significant phenotypic variability among individuals having identical genotypes and environments.

The stochastic variability that we observed among simulations can be detected early during the simulations. Figure 3 depicts the distribution of adherent (blue) cells no longer free to migrate and of nonadherent (yellow) cells still

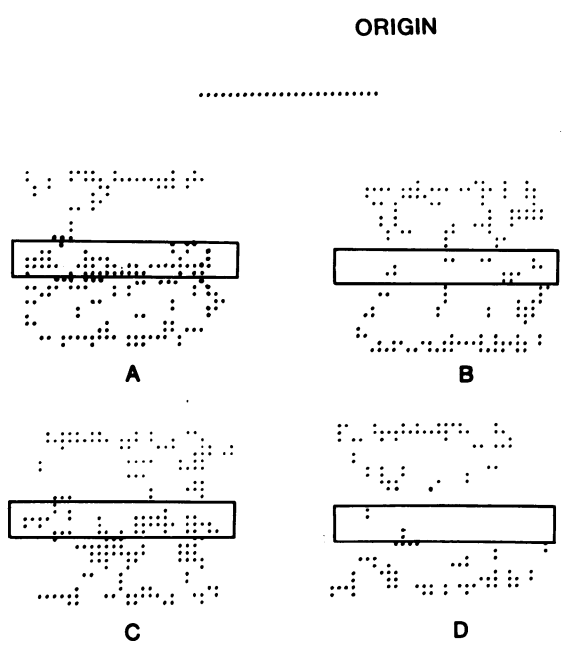


FIG. 1.—Stochastic effects on computer simulations for endocardial cushion-to-cushion fusion. The details and parameters of the computer simulations depicted are given in detail in Kurnit et al. (1985*a*). In brief, random-walking endocardial cells (starting as two lines corresponding to the two endocardial cushions) were allowed to migrate, divide, and adhere according to preselected probabilities for these options. The options were defined as (1) migration of one space in any one of four directions (up, down, left, or right), (2) cell division followed by migration of one of the daughter cells, and (3) quiescence, i.e., no migration or cell division. After a round in which every cell is afforded the opportunity to divide, migrate, or remain quiescent, any pair of cells that lies on adjacent nondiagonal grid cells was given a fixed probability to undergo cell-cell recognition and "adhere." Cells that adhered were no longer able to divide or migrate, although other cells could adhere to them. The process of allowing each cell to migrate and/or divide, followed by an examination of the grid for cell pairs that were then allowed to adhere, was iterated repetitively. In the simulations depicted above, the probabilities are as follows: migration = .7; division = .2; quiescence = .1; and adhesion, A, = .25. A gradient of migration was employed so that cells from the upper cushion were more likely to move downward and vice versa, as in the study of Kurnit et al. (1985*a*). The outlined rectangle represents the targeted area of cushion-to-cushion fusion (Kurnit et al. 1985*a*); as in the chick embryo (Hay and Low 1972), this region is packed more densely with cells from opposing cushions than are the surrounding regions. The same values for all parameters were used in each of the panels in fig. 1, so that the variations in outcome (i.e., the number of cells in the targeted rectangle) reflect stochastic differences due to the pseudorandom numbers generated during each of the independent probabilistic computer simulations depicted above.

free to migrate during simulations depicted in figure 2. Although large numbers of cells are involved in the simulation, outgrowth of the model endocardial cushions devolved from outgrowth of few clusters of nonadherent cells. The number, configuration, and longevity of this limited number of growth clusters were determined early during the course of these simulations when there were few endocardial cells. Panel *A* of figure 3 illustrates the growth clusters in a

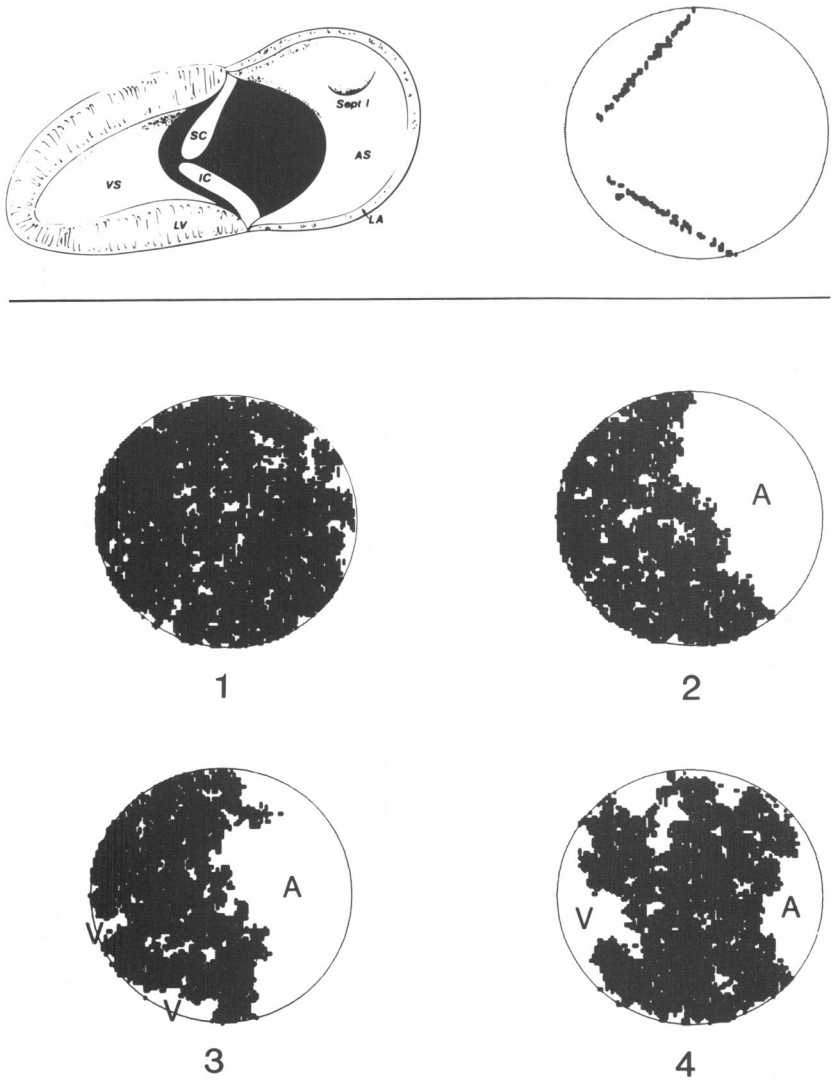
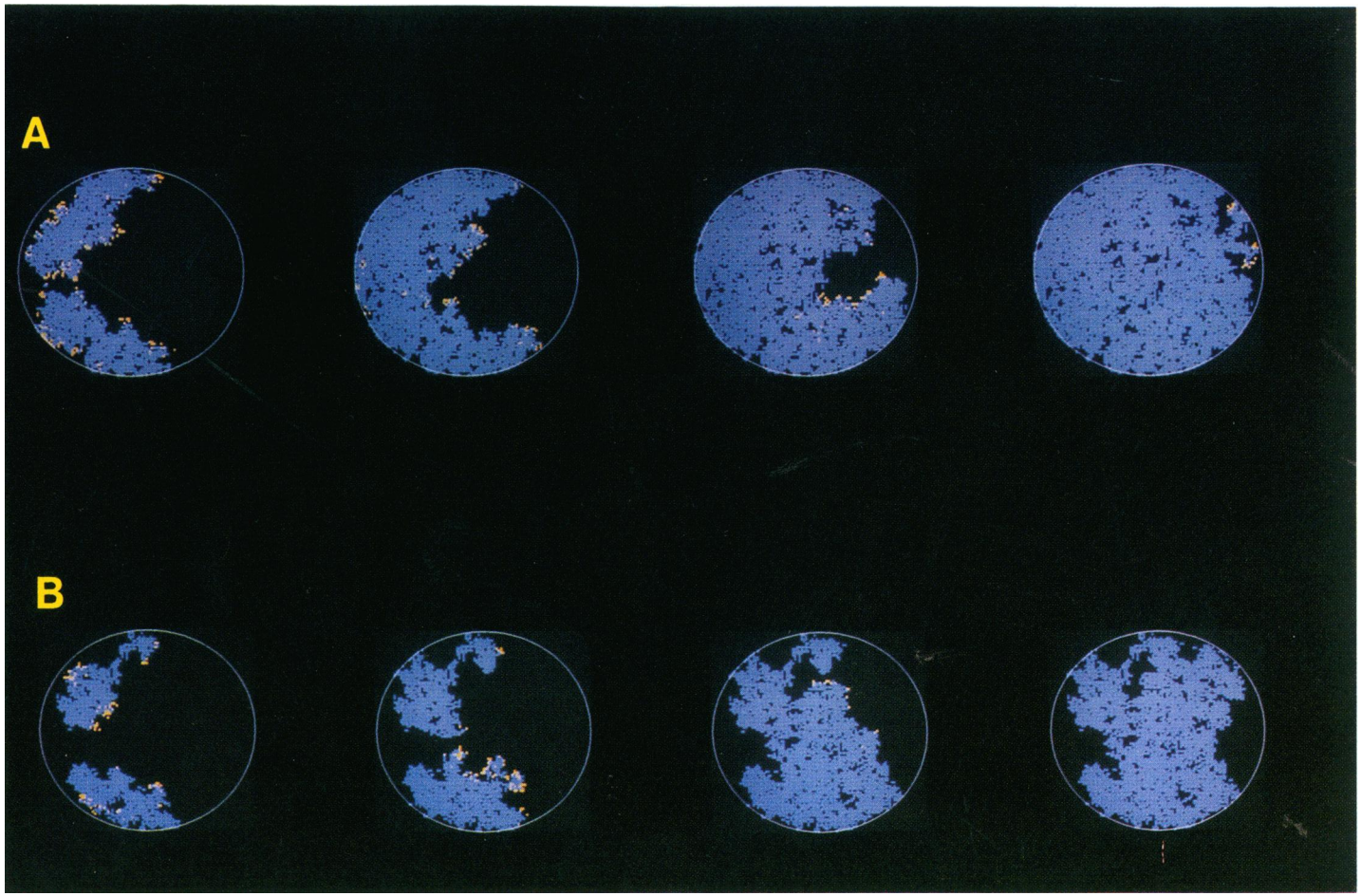


FIG. 2.—Stochastic effects on computer simulations for endocardial cushion-to-septum fusion. The drawing depicts the anatomy of the endocardial cushions in the AV canal, with an oblique orientation close to the muscular ventricular septum. VS = ventricular septum; SC = superior endocardial cushion; IC = inferior endocardial cushion; LV = left ventricle; LA = left atrium; AS = atrial septum; and Sept I = septum primum. The model portrays the AV canal as a circle to simplify the simulation. In the model, random-walking endocardial cells were allowed to migrate, divide, and adhere as in fig. 1. No gradient of migration was used. In each of the above four simulations, identical parameters were used, with probabilities as follows: division = .3; migration = .3; quiescence = .4; and adhesion = .1. Significant variability was observed, with phenotypes ranging from normal (no. *1*) to significant atrial (A) and ventricular (V) septal defects (nos. *2–4*).

simulation that yielded normal (complete) AV canal septation (fig. 2, simulation 1); panel *B* illustrates the growth clusters in a simulation that yielded abnormal (incomplete) septation (fig. 2, simulation 4). In these simulations, the final outgrowth of large numbers of cells depends sensitively on probabilistic interactions among small numbers of cells and growth clusters, thereby amplifying the effects of initial stochastic variations and introducing a significant role for chance.

Figure 4 demonstrates how the stochastic single-gene model can generate the family of continuous liability curves postulated in the multifactorial threshold model (Falconer 1965). We performed multiple independent iterations of the computer simulation for endocardial cushion-to-cushion fusion (fig. 1). Each of the 1,000 simulations summarized in a given panel of figure 4 used identical parameters, thereby defining equivalent genotypes and environments for each of the simulations within a panel. The abscissa represents the number of cells that were present in the targeted region of cushion-to-cushion fusion (the area inside the rectangles in fig. 1) after a given simulation ran to completion, and the ordinate represents the fraction of simulations that yielded the number of cells indicated on the abscissa. The variability observed among simulations in each panel must therefore be stochastic. This stochastic variability results from probabilistic interactions among small numbers of cells in early development (see fig. 3). As demonstrated by the curves in figure 4, this variability is intrinsic to the simulation, is distributed continuously, and does not require the invocation of outside factors such as other genetic changes or environmental influences. These simulations illustrate how morphogenesis can be represented as a game of chance.

As depicted in figure 4, the stochastic single-gene model can simulate a multifactorial threshold model. We considered the number of cells in the targeted area of endocardial cushion-to-cushion fusion (fig. 1) to represent intactness of the modeled endocardial septum. The presence of a threshold value of ≥ 25 cells in this targeted area was considered to be normal, whereas <25 cells was considered to represent an endocardial cushion defect. Since single genes can control intercellular adhesiveness (Edelman 1984), we modeled single-gene changes by altering a single parameter in our simulations, viz., the value for intercellular adhesiveness. Thus, the only parameter that distinguishes each of the panels in figure 4 is the adhesiveness value (denoted as A in fig. 4). As the adhesiveness value is increased, the liability curves are shifted to the left, yielding an increased risk to develop an endocardial cushion defect. Two salient points emerge. First, the family of curves resembles the continuously distributed normal liability curves of the multifactorial threshold model. Although the curves in figure 4 need not be normal to conform to the multifactorial model of Falconer (1965), most of the curves in figure 4 indeed pass a Kolmogorov-Smirnov goodness-of-fit test for normality. Second, an irreducible residuum of uncertainty remains: knowledge of both an individual's genotype and environment does not guarantee whether an individual will manifest a defect, although it does predict the probability (risk) of manifesting the defect.


A
B

RECURRENCE RISKS PREDICTED BY THE STOCHASTIC SINGLE-GENE MODEL

Figure 5 depicts the recurrence risks expected from a stochastic monogenic threshold model. As proposed by James (1971), assume a two-allele model in Hardy-Weinberg equilibrium, with the wild-type allele denoted A and the mutant allele denoted B. Let the gene frequency for A = a, and the gene frequency for B = $1 - a = b$ (see Appendix for more detailed discussion). As depicted in figure 5, the general features of this model are similar to those described by polygenic inheritance models: given an affected index case, the risk to his or her first-degree relatives is considerably above the background level (the risk in the general population), and this risk decreases as the relationship becomes more remote, approaching the background level. Since mutant homozygotes are at greater risk than heterozygotes to manifest the abnormal phenotype (James's model includes a dominance-variance component), the risk to siblings may be greater than the risk to other first-degree relatives, as has been observed in some clinical studies (Carter 1965; Nora 1968).

EPIDEMIOLOGY OF CARDIAC-SEPTAL DEFECTS

In Down syndrome, most congenital heart defects represent an AV canal defect due to abnormal outgrowth of tissues that relate to the endocardial cushions (for review, see Kurnit et al. 1985*a*). Significant variability occurs, with ~40% of individuals with Down syndrome manifesting overt congenital heart defects. These malformations include complete, ventral (membranous-type VSD), and dorsal (atrial septal defects) AV canal defects. Even Down syndrome subjects who do not have a clinically significant defect may manifest a forme fruste characterized by enlargement of the membranous ventricular septum relative to the muscularized ventricular septum (Rosenquist et al. 1974). Discordance for congenital heart defects between monozygous twins with Down syndrome has been noted (Rehder 1981). In the murine trisomy 16 model for human trisomy 21, inbred trisomy 16 littermates are also discordant for congenital heart defects (Miyabara et al. 1984), a result that again documents the importance of stochastic effects.

A recent comprehensive survey (Newman 1985) of the epidemiology of VSD supported the conclusion that chance plays a major role in the etiology of VSD in man. Incidence rates for VSD are similar in different races and seasons and

FIG. 3.—Instability of growth processes during endocardial cushion outgrowth. The pattern of endocardial cushion outgrowth for two of the simulations depicted in fig. 2 is shown, with adherent cells (that may no longer migrate or divide) indicated in blue and nonadherent cells (still free to migrate and/or divide) indicated in yellow. The patterns are shown at 50, 100, 200, and 300 iterations of the program, as defined in fig. 1. The final result is obtained when all cells are nonadherent (blue). Panel *A* shows the time course of cushion outgrowth for the simulation (fig. 2, no. *1*) that gave normal, complete cushion outgrowth; panel *B* shows the analogous time course for the simulation (fig. 2, no. *4*) that gave abnormal cushion outgrowth with both atrial and ventricular septal defects. Inspection shows that the abnormal simulation results from the early extinction of clusters of nonadherent (yellow) cells or from the encirclement of such clusters by adherent (blue) cells; in contrast, the normal simulation results from the continued presence and activity of these clusters of nonadherent (yellow) cells.

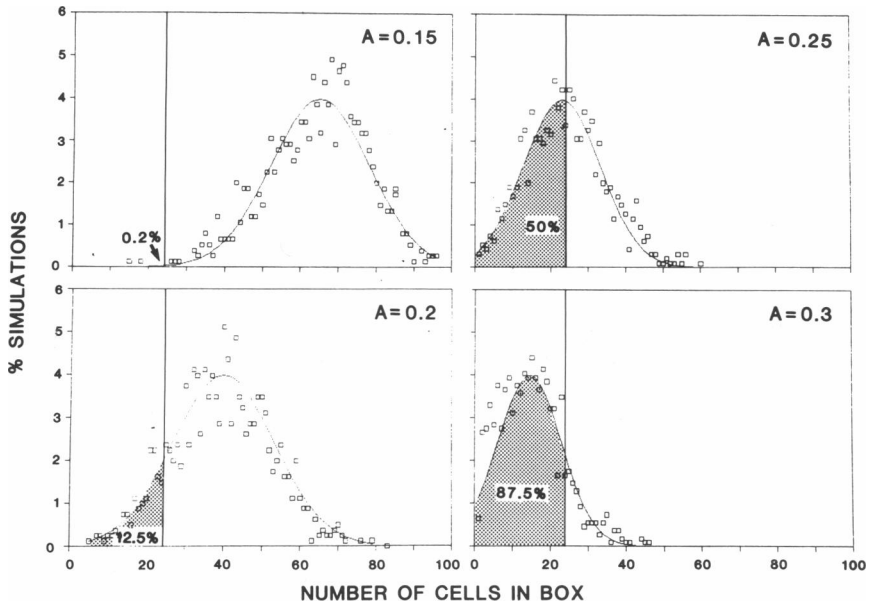


FIG. 4.—Threshold model for stochastic monogenic inheritance. The computer simulations that depict endocardial cushion-to-cushion fusion (fig. 1) were run 1,000 times apiece for a given set of parameters. The number of cells in the targeted rectangle (representing the region of cushion-to-cushion fusion) was counted at the end of each simulation, when no nonadherent cells remained. Each panel depicts the tabulation for this number of cells following multiple simulations using a given set of parameters. The abscissa gives the number of cells in the targeted rectangle, and the ordinate gives the fraction of simulations using a given set of parameters that yielded that number of cells in the rectangle. The line perpendicular to the abscissa at 25 cells indicates the threshold value, below which it was judged that too few cells were present to yield a normal cushion-to-cushion fusion. All simulations in fig. 4 used the same values of migration, division, quiescence, and gradient used in fig. 1. In each panel, a different value of adhesiveness (A) was used. In each panel, the percentage of simulations that yield abnormal simulations with <25 cells in the targeted rectangle is given. As the adhesiveness is increased, there is a tendency for fewer cells to reach the targeted rectangle, increasing the probability of an endocardial cushion defect. Since only a single parameter, adhesiveness, is varied among the panels, the data shown here exemplify a single-gene stochastic model. To test whether the curves conformed to a normal distribution, we employed a Kolmogorov-Smirnov test (Sokol 1981). The curves for A = 0.15, A = 0.20, and A = 0.25 did not differ significantly from the normal distribution when this test was used ($P > .05$); the curve for A = 0.3 was truncated, so that application of this test was not appropriate.

do not correlate with maternal age, birth order, sex, or socioeconomic status. Few cases are attributable to teratogens. Although genetic factors are implicated by a recurrence risk of between 1% and 5% for congenital heart disease in first-degree relatives (summarized in Newman 1985), most VSD are not associated with recognized Mendelian or chromosomal syndromes. Further support for the role of stochastic effects comes from the finding that monozygotic twins, who share identical genotypes and similar prenatal environments, show a high discordance rate (only 2/26 concordant) for VSD (Newman 1985).

A classic example of the interaction between a single-gene mutation and chance is the existence of murine and human autosomal recessive single-gene

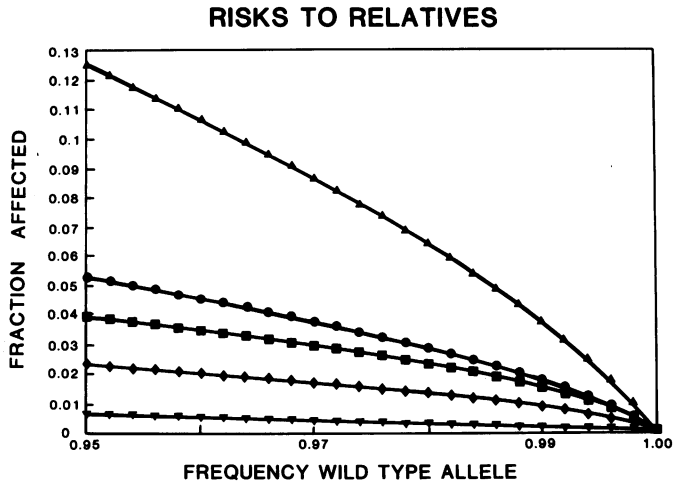


FIG. 5.—Population structure derived from single-gene stochastic inheritance model. Depicted are the risks to relatives of an index case whose genotype is unknown. The risks were calculated from the model of James (1971), as described by the equations in the Appendix. The probability that a given genotype shows the abnormal phenotype was set as follows: P_0 (*aa*, wild-type homozygote) = .001; P_1 (*ab*, heterozygote) = .05; P_2 (*bb*, mutant homozygote) = .5. Symbols denote risk for the general population (▼), second-generation ancestor or descendant (◆), first-generation ancestor or descendant (■), sibling other than monozygous twin (●), and monozygous twin (▶).

mutations that result in random determination of situs, so that 50% of mutants homozygous for the mutant gene manifest situs inversus (fig. 6) (Kartagener 1933; Feldman 1935; Cockayne 1938; Ivemark 1955; Afzelius 1976; Layton 1976; Arnold et al. 1983; Niikawa et al. 1983). As illustrated by the murine *iv/iv* (Hummel and Chapman 1959; Layton 1976; Layton and Dupree, in press) mutant, the salient features consistent with a stochastic single-gene model are as follows: (1) A single mutant gene is responsible for the abnormal phenotype. (2) Chance plays a major role, as evidenced by the coin-flip determination of laterality. (3) Other malformations, including AV canal defects, are present in a significant plurality of animals but are not present in all animals. These features have all remained true following the crossing of the *iv/iv* mutation onto an inbred C57BL/6 background, so that phenotypic variability due to chance persists among littermates having identical genotypes and uterine environments.

Thus, in a number of cases all cardiac septal defects involve interactions between chance and genes. In the *iv/iv* mouse, a single gene is involved. In the more general case of congenital heart disease in the population, the epidemiology (Newman 1985) is compatible with that described by the stochastic single-gene model, with an incidence of 1%–5% in first-degree relatives and 8% concordance between monozygotic twins. (In advocating a single-gene model, we do not imply that a single gene is responsible for all VSD; rather we imply that, within a given family, mutation at a single locus may be responsible for the predisposition to VSD. As discussed below, specific single-gene mutations in given families may be associated with different risks.) In Down syndrome, we have shown that abnormal intercellular adhesiveness exists (Wright et al. 1984)

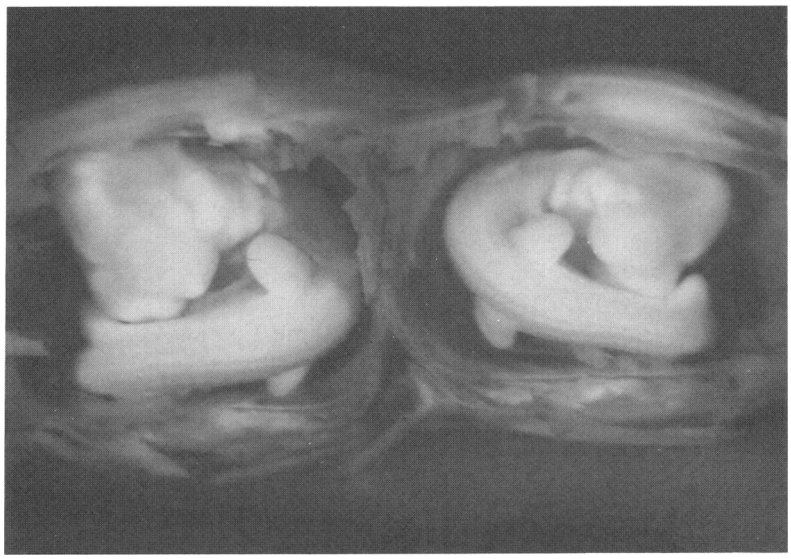


FIG. 6.—Random determination of laterality in *iv/iv* mice. Two 10-day embryonic littermates with identical *iv/iv* genotypes are shown: the mouse on the left manifests normal (right-handed) coiling, whereas its littermate shows a left-handed coil. Reprinted from Layton (1976) (© 1976 by the American Genetic Association).

and might underlie the occurrence of VSD in this disorder, based on dosage for gene(s) encoded by chromosome 21 that effect adhesiveness of endocardial cells (Kurnit et al. 1985*a*). In some cases, VSD may occur by chance without genetic predisposition. For example, ectopia cordis (which does not show familial clustering and is associated with heart defects including VSD [Van Praagh et al. 1977]) results from a developmental accident at 3 wk gestation (Kaplan et al. 1985). All these findings indicate that both genetics and chance are causal in the etiology of cardiac septal defects.

EVIDENCE FOR THE STOCHASTIC MODEL IN NATURE

The figures in the present article illustrate how chance plays a role in cardiac malformations; we note that the principle is more general, since stochastic models can explain a number of developmental phenomena, including cell growth (Smith and Martin 1973; Rabinovitch 1983), aging (Holliday et al. 1977; Smith and Whitney 1980), hemopoiesis (Till et al. 1964; Gusella et al. 1976; Stamatoyannopoulos et al. 1981; Suda et al. 1984; Kurnit et al. 1985*b*), differentiation of B lymphocytes (Coleclough 1983), and oncogenesis (Knudson 1971; Moolgavkar and Venzon 1979). In particular, the molecular basis for the stochastic event in retinoblastoma has been elucidated: it is the occurrence of a gene conversion or mitotic crossover in cells previously heterozygous at a single locus that results in homozygosity for the abnormal allele (Cavenee et al. 1983). The latter case illustrates that a single-gene stochastic model can explain

the occurrence of retinoblastoma, and the model appears to be applicable to other cancers as well (Dryja et al. 1984; Solomon 1984; Burt et al. 1985; Koufos et al. 1985).

STOCHASTIC SINGLE-GENE MODEL: COMPARISON BETWEEN OBSERVED AND PREDICTED DATA

The epidemiology of malformations not associated with recognized Mendelian syndromes has several features that must be explicable under any model purporting to explain non-Mendelian transmission. We detail how the stochastic single-gene model can explain these features as follows:

1. In general, recurrence risk increases with the number of affected relatives (Edwards 1960), with the severity of the defect in the index case, and with whether the index case occurs in a group (e.g., a particular sex or ethnic group) in which the malformation is less commonly seen. In the stochastic single-gene model, as in the polygenic model, such cases would likely have a more abnormal genotype whose risk curve (figs. 4, 5) is shifted further away from the norm. In the single-gene model, such cases with a more abnormal genotype would correspond to homozygotes for an abnormal allele, to the more abnormal allele of a series of mutant alleles at a single locus, and/or to a single mutant gene locus associated with higher risks than are other mutant loci in different families.

2. Single-gene Mendelian syndromes are recognized on the basis of phenotypes, which are often complex. Most such recognizable syndromes (Smith 1982) consist of a constellation of features, any one of which is present in only a fraction of those with the disorder. Thus, stochastic factors operate in Mendelian malformation syndromes as well, resulting in the variability with which different features of the syndrome are found in different affected individuals, even in members of the same kindred in whom the genetic abnormality at the affected locus is identical. In this light, Mendelian syndromes can be considered as a special case of the stochastic single-gene model. If this model is assumed, Mendelian transmission of a syndrome ensues either from a high probability of manifesting one or a few traits or from moderate probabilities of manifesting any of a larger number of traits. To identify less "penetrant" single-gene syndromes (figs. 4, 5), one should search for phenotypic markers that are manifested with higher probabilities than the clinical condition itself (e.g., use both abnormal eye movements and psychotic behavior to ascertain gene carriers for a putative single allele for schizophrenia [Matthysse et al. 1986], establish linkage to DNA markers [Botstein et al. 1980] as in familial Alzheimer's disease [St. George-Hyslop et al. 1987], or search for premalignant lesions such as adenomatous colonic polyps in familial colorectal cancer pedigrees [Burt et al. 1985]). Mathematical methods are available for analyzing latent Mendelian traits with a number of possible manifestations (Matthysse et al. 1986).

3. A criticism that might be directed at the single-gene model in figure 5 is that the recurrence risk does not fall off steeply enough among successive

generations (Carter 1965). The introduction of selection effects and/or an oligogenic model invoking a few major loci (Hopkinson et al. 1964) can yield such steeper gradients.

4. In sum, the single-gene stochastic model can satisfy the specifications for which polygenic models were elaborated. Indeed, the single-gene stochastic model is more flexible, since the upper boundaries for recurrence risk that are inherent in polygenic models (Edwards 1969; Smith 1971) do not apply.

THE ROLE OF CHANCE IN DEVELOPMENT

The concepts that we outline should be applicable to a wide variety of events in morphogenesis. Details of the mechanics of the model are not critical. For example, other computer simulation models (Fraser 1980; Bodenstein, in press) that made very different assumptions about how migration and adhesiveness might affect morphogenesis also yielded significant variability on a stochastic basis. Inorganic growth processes determined on the basis of surface tension and diffusion may also be simulated by an irreversible stochastic model (Rikvold 1982). Thus, although our simulations were meant to model cardiogenesis, the concepts that we outline should apply to any event in which small numbers of units interact, in probabilistic fashion, in a way that influences later masses involving larger aggregates (see fig. 3). Since an 8-day mouse embryo has $\sim 10^4$ (Ozato et al. 1985) cells, and since our simulations (figs. 2, 3) involved as many as 10^4 cells, the embryo and its constituent organs do indeed contain a sufficiently small number of cells at critical times of organogenesis.

A major concept that is evident in our simulations is that randomness devolves from *sensitive dependence on initial conditions and events*. In traditional classical mechanics, two objects that start close together and obey the same deterministic laws will remain near each other over time. In contrast, in many common dynamical systems, it is now known that, however close the starting points of two objects, they may eventually be found far apart (Ruelle 1980). Thus, even a deterministic system may behave in a chaotic or unpredictable way. Dynamical systems sensitive to initial conditions and/or small perturbations early during their evolution (see fig. 3) will amplify stochastic events, so that the eventual outcome may, as in our simulations, evidence randomness (Lorenz 1963; Ruelle 1980).

In our view, randomness is inherent in embryology, not merely an artifact of the incomplete state of our understanding of developmental processes. It is not a fundamental randomness in the quantum-mechanical sense, i.e., an uncertainty that escapes all possible reduction by measurement. If the positions and characteristics of every cell in the developing embryo were known, it would be possible *in principle* to predict its development, and there would be no randomness in outcome. Rather, biologic uncertainty ensues from sensitivity to early perturbations: if the state of the embryonic system is described at the level of detail proper to biology, small but significant fluctuations will occur from embryo to embryo; amplification of these fluctuations (see fig. 3) yields an element of randomness in developmental outcome that cannot be escaped.

IMPLICATIONS FOR CLINICAL GENETICS

We have presented evidence that several factors play a role in the occurrence of phenotypic differences, including such differences as inborn errors of morphogenesis: *genetics, environment,* and *chance* all play a role in the expression of a given phenotype. We emphasize three concepts. First, chance is a significant factor in development. Second, multifactorial patterns of inheritance may be explained by single-gene models as well as by polygenic models. Third, even if it becomes feasible to predict and/or control both genotype and environment during pregnancy, birth defects due to chance will still occur.

ACKNOWLEDGMENT

Research support was provided by National Institutes of Health grants R01 HD20118 and HL37703. D.M.K. is an Investigator at the Howard Hughes Medical Institute, Ann Arbor, MI.

APPENDIX

Assume Hardy-Weinberg equilibrium for a two-allele autosomal locus with gene frequency = a for the wild-type allele (A), and gene frequency = b for the mutant allele (B). The probability that an individual with genotype AA shows the abnormal phenotype is P_0; the probability that the genotype AB shows the abnormal phenotype is P_1; the probability that the genotype BB shows the abnormal phenotype is P_2.

The probability that any individual will manifest an abnormal phenotype = (P_0) (probability that an individual has genotype AA) + (P_1) (probability that an individual has genotype AB) + (P_2) (probability that an individual has genotype BB).

The incidence, x, of the phenotypic abnormality in the general population is $x = P_0a^2 + 2P_1ab + P_2b^2$. The fraction of affected cases due to each genotype is as follows: Fraction due to AA = P_0a^2/x; fraction due to AB = $2P_1ab/x$; and fraction due to BB = P_2b^2/x.

The probability that an nth-degree descendant or ancestor of an index case will manifest the malformation is greater than the incidence in the general population. The calculations for inheritance by descent are analogous to those of Li and Sachs (1954). If the index case is a homozygote (AA or BB), then the probability that an nth-generation descendant has inherited the given allele from the index case by descent is $(\frac{1}{2})^{n-1}$. If the index case is a heterozygote, then the probability that an nth-generation descendant has inherited the A allele from the heterozygous index case by descent is $(\frac{1}{2})^n$; analogously, the probability that an nth-generation descendant has inherited the B allele from the heterozygous index case by descent is also $(\frac{1}{2})^n$. For the case in which the chromosome is inherited from the index case or descendants, the probability that an nth-generation descendant inherits the allele on that chromosome from a family member other than the index case is $1 - (\frac{1}{2})^{n-1}$; in this event, the probability of inheriting allele A = a and the probability of inheriting allele B = b, reflecting the frequency of these alleles in the general population. For the case in which the chromosome is inherited through the parent not descended from the index case, the probability of inheriting allele A = a and the probability of inheriting allele B = b, again reflecting the frequency of these alleles in the general population.

The probability, P, that an nth-generation descendant of an index case will be affected is determined for each of the three types of index cases (i.e., the index case is AA, AB,

or BB). For each of the three cases, the probability that the nth-generation relative will have a given genotype, and thence an abnormal phenotype, is determined on the basis of the above considerations. For each of the three cases, this probability is then multiplied by the fraction of index cases that carry the specified genotype. Summation of the probabilities for all three cases then yields P.

For the special case of siblings, without a priori knowledge of the parents' genotypes, the following considerations apply: (1) If the index case has genotype AA, then the probability that a sib has genotype AA $= (1 + a)^2/4$, that the sib has genotype BB $= b^2/4$, or that the sib has genotype AB $= 1 - (1 + a)^2/4 - b^2/4$. (2) If the index case has genotype BB, then the probability that a sib has genotype BB $= (1 + b)^2/4$, that the sib has genotype AA $= a^2/4$, or that the sib has genotype AB $= 1 - (1 + b)^2/4 - a^2/4$. (3) If the index case has genotype AB, then the probability that a sib has genotype AA $= (a + a^2)/4$, that the sib has genotype BB $= (b + b^2)/4$, or that the sib has genotype AB $= 1 - (a + a^2)/4 - (b + b^2)/4$.

For the special case of monozygotic twins, both twins have identical genotypes.

If all of the above considerations are taken into account, the following results occur: (1) The probability, P, that an index case's monozygotic twin will manifest the abnormal phenotype is $P_0^2a^2/x + 2P_1^2ab/x + P_2^2b^2/x$. (2) The probability, P, that an index case's first-generation relative (other than siblings) will manifest the abnormal phenotype is

$$\frac{P_2b^2}{x}(aP_1 + bP_2) + \frac{2P_1ab}{x}[a/2(P_1 + P_0) + b/2(P_2 + P_1)] + \frac{P_0a^2}{x}(aP_0 + bP_1)$$

If the above relation is generalized, the probability, P, that an index case's nth-generation relative (other than siblings) will manifest the abnormal phenotype is as follows:

$$\begin{aligned}
&\frac{P_2b^2}{x}a[(\tfrac{1}{2})^{n-1}(P_1) + (1 - (\tfrac{1}{2})^{n-1})(aP_0 + bP_1)] \\
&\quad + \frac{P_2b^2}{x}b\{(\tfrac{1}{2})^{n-1}(P_2) + [1 - (\tfrac{1}{2})^{n-1}](aP_1 + bP_2)\} \\
&\quad + \frac{2P_1ab}{x}a\{(\tfrac{1}{2})^nP_1 + (\tfrac{1}{2})^nP_0 + [1 - (\tfrac{1}{2})^{n-1}](aP_0 + bP_1)\} \\
&\quad + \frac{2P_1ab}{x}b\{(\tfrac{1}{2})^nP_2 + (\tfrac{1}{2})^nP_1 + [1 - (\tfrac{1}{2})^{n-1}](aP_1 + bP_2)\} \\
&\quad + \frac{P_0a^2}{x}a[(\tfrac{1}{2})^{n-1}(P_0) + (1 - (\tfrac{1}{2})^{n-1})(aP_0 + bP_1)] \\
&\quad + \frac{P_0a^2}{x}b\{(\tfrac{1}{2})^{n-1}(P_1) + [1 - (\tfrac{1}{2})^{n-1}](aP_1 + bP_2)\} .
\end{aligned}$$

The probability, P, that an index case's sibling will manifest the abnormal phenotype is as follows:

$$\frac{P_0a^2}{4x}[P_0(1+a)^2 + P_1z + P_2b^2]$$

$$+ \frac{P_1ab}{2x}[P_0(a+a^2) + P_1(3-a^2-b^2) + P_2(b+b^2)]$$

$$+ \frac{P_2b^2}{4x}[P_0a^2 + P_1y + P_2(1+b)^2] ,$$

where $z = 4 - (1+a)^2 - b^2$, and $y = 4 - (1+b)^2 - a^2$.

REFERENCES

Afzelius, B. A. 1976. A human syndrome caused by immotile cilia. Science **193**:317–319.

Arnold, G. L., D. Bixler, and D. Girod. 1983. Probable autosomal recessive inheritance of polysplenia, situs inversus and cardiac defects in an Amish family. Am. J. Med. Genet. **16**:35–42.

Bodenstein, L. A dynamic simulation model of tissue growth and cell patterning. Cell Differ. (in press).

Botstein, D., R. L. White, M. Skolnick, and R. W. Davis. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. **32**:314–331.

Burt, R. W., T. Bishop, L. A. Cannon, M. A. Dowdle, R. G. Lee, and M. H. Skolnick. 1985. Dominant inheritance of adenomatous colonic polyps and colorectal cancer. N. Engl. J. Med. **312**:1540–1544.

Carter, C. O. 1965. The inheritance of common congenital malformations. Prog. Med. Genet. **4**:59–84.

Cavenee, W. K., T. P. Dryja, R. A. Phillips, W. F. Benedict, R. Godbout, B. L. Gallie, A. L. Murphree, L. C. Strong, and R. L. White. 1983. Expression of recessive alleles by chromosomal mechanisms in retinoblastoma. Nature **305**:779–784.

Cockayne, E. A. 1938. The genetics of transposition of the vicera. Q. J. Med. **31**:479–493.

Coleclough, C. 1983. Chance, necessity and antibody gene dynamics. Nature **303**:23–26.

Dryja, T. P., W. Cavenee, R. White, J. M. Rapaport, R. Petersen, D. M. Albert, and G. A. P. Bruns. 1984. Homozygosity of chromosome 13 in retinoblastoma. N. Engl. J. Med. **310**:550–553.

Edelman, G. 1984. Cell adhesion and morphogenesis: the regulator hypothesis. Proc. Natl. Acad. Sci. USA **81**:1460–1464.

Edwards, J. H. 1960. The simulation of Mendelism. Acta Genet. **10**:63–70.

———. 1969. Familial predisposition in man. Br. Med. Bull. **25**:58–63.

Falconer, D. S. 1965. The inheritance of liability to certain diseases, estimated from the incidence among relatives. Ann. Hum. Genet. **29**:51–71.

Feldman, W. 1935. Transposition of the viscera behaving as a Mendelian recessive with congenital absence of the appendix. Proc. R. Soc. Med. (Lond.) **28**:753–756.

Fraser, S. E. 1980. A differential adhesion approach to the patterning of nerve connections. Dev. Biol. **79**:453–464.

Gusella, J., R. Geller, B. Clarke, V. Weeks, and D. Housman. 1976. Commitment to erythroid differentiation by Friend leukemia cells: a stochastic analysis. Cell **9**:221–229.

Hay, D. A., and F. N. Low. 1972. The fusion of dorsal and ventral endocardial cushions in the embryonic chick heart. Am. J. Anat. **133**:1–24.

Holliday, R., L. I. Huschtscha, G. M. Tarrant, and T. B. L. Kirkwood. 1977. Testing the commitment theory of aging. Science **198**:366–372.

Hopkinson, D. A., N. Spencer, and H. Harris. 1964. Genetical studies on human red cell acid phosphatase. Am. J. Hum. Genet. **16**:141–154.

Hummel, K. P., and D. B. Chapman. 1959. Visceral inversion and associated anomalies in the mouse. J. Hered. **50**:9–13.

Ivemark, B. I. 1955. Implications of agenesis of the spleen on the pathogenesis of conotruncus anomalies in childhood. Acta Paediatr. Scand. **44**[Suppl. 104]: 590–592.

James, J. W. 1971. Frequency in relatives for an all-or-none trait. Ann. Hum. Genet. **35**:47–49:

Kaplan, L. C., R. Matsuoka, E. F. Gilbert, J. M. Opitz, and D. M. Kurnit. 1985. Ectopia cordis and cleft sternum: evidence for mechanical teratogenesis following rupture of the chorion or yolk sac. Am. J. Med. Genet. **21**:187–199.

Kartagener, M. 1933. Zur Pathogenese der Bronchiektasien: Bronchiektasien bei Situs Viscerus Inversus. Beitr. Klin. Tuberk. **83**:489–501.

Knudson, A. G., Jr. 1971. Mutation and cancer: statistical study of retinoblastoma. Proc. Natl. Acad. Sci. USA **68**:820–823.

Koufos, A., M. F. Hansen, N. G. Copeland, N. A. Jenkins, B. C. Lampkin, and W. K. Cavenee. 1985. Loss of heterozygosity in three embryonal tumors suggests a common pathogenetic mechanism. Nature **316**:330–334.

Kurnit, D. M., J. F. Aldridge, R. Matsuoka, and S. Matthysse. 1985*a*. Increased adhesiveness of trisomy 21 cells and atrioventricular canal malformations in Down syndrome: a stochastic model. Am. J. Med. Genet. **30**:385–399.

Kurnit, D. M., S. Matthysse, T. Papayannopoulou, and G. Stamatoyannopoulos. 1985*b*. Stochastic branching model for hemopoietic progenitor cell differentiation. J. Cell. Physiol. **123**:55–63.

Layton, W. M. 1976. Random determination of a developmental process: reversal of normal visceral asymmetry in the mouse. J. Hered. **67**:336–338.

Layton, W. M., and W. B. Dupree. An animal model for polysplenia, asplenia, and other syndromes characterized by heterotaxia and heart malformations. Am. J. Cardiol. (in press).

Li, C. C., and L. Sachs. 1954. The derivation of joint distribution and correlation by the use of stochastic matrices. Biometrics **10**:347–360.

Lorenz, E. N. 1963. Deterministic nonperiodic flow. J. Atmos. Sci. **20**:130–141.

Matthysse, S., P. Holzman, and K. Lange. 1986. The genetic transmission of schizophrenia: application of Mendelian latent structure analysis to eye tracking dysfunctions in schizophrenia and affective disorder. J. Psychiatr. Res. **20**:57–76.

Miyabara, S., H. Sugihara, N. Yonemitsu, and K. Yun. 1984. Comparative study of the phenotypic expression of mice trisomy 16 by different female strains: attempt at an animal model for human trisomy 21. Congenital Anomalies **24**:283–292.

Moolgavkar, S. H., and D. E. Venzon. 1979. Two-event models for carcinogenesis: incidence curves for childhood and adult tumors. Math. Biosci. **47**:55–77.

Newman, T. B. 1985. Etiology of ventricular septal defects: an epidemiologic approach. Pediatrics **76**:741–749.

Niikawa, N., S. Kohsaka, M. Mizumoto, I. Hamada, and T. Kajii. 1983. Familial clustering of situs inversus totalis and asplenia and polysplenia syndromes. Am. J. Med. Genet. **16**:43–47.

Nora, J. J. 1968. Multifactorial inheritance hypothesis for the etiology of congenital heart diseases: the genetic-environmental interaction. Circulation **38**:604–617.

Ozato, K., Y.-J. Wan, and B. Orrison. 1985. Mouse major histocompatibility class I gene expression begins at the midsomite stage and is inducible in earlier-stage embryos by interferon. Proc. Natl. Acad. Sci. USA **82**:2427–2431.

Penrose, L. 1953. The genetical background of common diseases. Acta Genet. **4**:257–265.

Rabinovitch, P. S. 1983. Regulation of human fibroblast growth rate by both noncycling cell fraction and transition probability is shown by growth in 5-bromodeoxyuridine followed by Hoechst 33258 flow cytometry. Proc. Natl. Acad. Sci. USA **80**:2951–2955.

Rehder, H. 1981. Pathology of trisomy 21—with particular reference to persistent common atrioventricular canal of the heart. Pp. 57–73 *in* G. R. Burgio, M. Fraccaro, L. Tiepolo and U. Wolf, eds. Trisomy 21: an international symposium, Rapallo, Italy, Nov. 8–10. Springer, Berlin.

Rikvold, P. A. 1982. Simulations of a stochastic model for cluster growth on a square lattice. Phys. Rev. **26**:647–650.

Rosenquist, G. C., L. J. Sweeney, J. Amsel, and H. A. McAllister. 1974. Enlargement of the membranous ventricular septum: an internal stigma of Down's syndrome. J. Pediatr. **85**:490–493.

Ruelle, D. 1980. Strange attractors. Math. Intelligencer **2**:126–137.

St. George-Hyslop, P., R. Tanzi, R. J. Polinsky, J. L. Haines, L. Nee, P. C. Watkins, R. Myers, R. Feldman, D. Pollen, D. Drachman, J. Growdon, A. Bruni, J.-F. Foncin, P. Frommelt, L. Amaducci, S. Sorbi, S. Piancentini, G. D. Stewart, W. Hobbs, P. M. Conneally, and J. F. Gusella. 1987. The genetic defect causing familiar Alzheimer's diasease maps on chromosome 21. Science **235**:885–890.

Smith, C. 1971. Recurrence risks for multifactorial inheritance. Am. J. Hum. Genet. **23**:578–588.

Smith, D. W. 1982. Recognizable patterns of human malformation. 3d ed. W. B. Saunders, Philadelphia.

Smith, J. A., and L. Martin. 1973. Do cells cycle? Proc. Natl. Acad. Sci. USA **70**:1263–1267.

Smith, J. R., and R. G. Whitney. 1980. Intraclonal variation in proliferative potential of human diploid fibroblasts: stochastic mechanism for cellular aging. Science **207**:82–84.

Sokol, R. 1981. Statistical tables. 2d ed. W. H. Freeman, San Francisco.

Solomon, E. 1984. Recessive mutation in aetiology of Wilm's tumor. Nature **309**:111–112.

Stamatoyannopoulos, G., D. Kurnit, and T. Papayannopoulou. 1981. Stochastic expression of fetal hemoglobin in adult erythroid cells. Proc. Natl. Acad. Sci. USA **78**:7005–7009.

Suda, T., J. Suda, and M. Ogawa. 1984. Disparate differentiation in mouse hemopoietic colonies derived from paired progenitors. Proc. Natl. Acad. Sci. USA **81**:2520–2524.

Till, J. E., E. A. McCulloch, and L. A. Siminovitch. 1964. A stochastic model of stem cell proliferation, based on the growth of spleen colony-forming cells. Proc. Natl. Acad. Sci. USA **51**:29–36.

Van Praagh, R., P. M. Weinberg, R. Matsuoka, and S. Van Praagh. 1977. Malpositions of the heart. Pp. 422–458 *in* A. J. Moss, F. H. Adams, and G. C. Emmanouilides, eds. Heart disease in infants, children and adolescents. Williams & Wilkins, Baltimore.

Wright, T. C., R. W. Orkin, M. Destrempes, and D. M. Kurnit. 1984. Increased adhesiveness of Down syndrome fetal fibroblasts in vitro. Proc. Natl. Acad. Sci. USA **81**:2426–2430.